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The Antioxidants as Protectors of Host Stress Organ Injury in Mice Infected with *Plasmodium berghei*

A.J. ARIF[1], SUBHASH CHANDRA[1] and CHANAN SINGH[1]

A study has been made of counteracting the stress organ injury in *Plasmodium* infection by means of antioxidants on the premise that free radicals are responsible for causing the injury to stress organs. This was evidenced by drastically altered biochemical parameters in liver and spleen of the host in terms of elevated levels of lipid peroxides and xanthine oxidase (XO) activity, and a fall in superoxide dismutase activity coupled with other drastic biochemical changes. The cardinal factor responsible for the above was considered to be XO which engenders free radicals purportedly responsible for the stress organ (biochemical) injury. Results demonstrate a lowering of lipid peroxide levels, xanthine oxidase activity, liver weight and modulation of protein level in liver of the host (mouse) in *Plasmodium* infection when treated with catechin, glutathione and propylgallate.

INTRODUCTION

Plasmodium infection has been shown to cause a tremendous increase in the xanthine oxidase (XO) (Singh *et al.*, 1985) activity in the host stress organs, and it concomitantly brings about serious biochemical alterations in the latter (Sharma *et al.*, 1978a; 1979), attributable to (XO) and other free radical generating systems (Chance *et al.*, 1979; Pryor, 1976; Ciba Geigy Foundation, 1979). Since an earlier attempt to mitigate/counteract the stress organ damage at molecular level against the free radicals had proved promising (Singh *et al.*, 1985) it was considered appropriate to apply an alternative approach consisting of the use of antioxidants for this purpose.

Accordingly, the present communication reports results of such a study, which corroborates the guiding concept, by demonstrating the ability of antioxidants to mitigate the stress organ injury in *Plasmodium* infection.

MATERIAL AND METHODS

The antioxidants (AOs), propylgallate (PRG), catechin (CAT), glutathione (GSH) and diphenyl-furan (DPF) (Sigma USA), were dissolved in sterile normal saline and administered subcutaneously. They were found to be safe upto a single dose of 15 mg/kg body weight of the animal (male mouse, 18-20 gm body weight at about six weeks of age).

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Table 1. Protection of the stress organ liver by antioxidants against undesirable biochemical alterations and injury at molecular level in Plasmodial infection

(i) Liver Weights (in gms)

Antioxidants	Treatment/Infection				Results of significance		
	UU	UT	IU	IT	UU/UT	UU/IU	UU/IT
Propylgallate (PRG)	1.36 ± 0.06	1.37 ± 0.09	2.03 ± 0.14	1.58 ± 0.04	NS	**↑↑	*↑
Catechin (CAT)	1.35 ± 0.07	1.44 ± 0.04	2.07 ± 0.08	1.54 ± 0.05	NS	**↑↑	*↑
Glutathione (GSH)	1.49 ± 0.05	1.55 ± 0.05	2.07 ± 0.09	1.58 ± 0.05	NS	**↑↑	NS
Diphenyl-furan (DF)	1.47 ± 0.04	1.54 ± 0.05	2.89 ± 0.13	2.03 ± 0.14	NS	**↑↑	**↑↑
	SE estimate ± 0.073						
(ii) Lipid Peroxides (MDA/ 100 gms wet liver)							
PRG	52.28 ± 1.24	50.15 ± 2.44	75.24 ± 1.36	53.83 ± 1.06	NS	**↑↑	NS
CAT	52.73 ± 1.28	48.85 ± 1.60	74.40 ± 1.35	54.97 ± 1.37	NS	**↑↑	NS
GSH	52.33 ± 1.24	49.04 ± 0.70	70.78 ± 1.38	57.04 ± 2.27	NS	**↑↑	NS
DF	50.00 ± 0.62	44.73 ± 1.69	76.45 ± 1.36	72.73 ± 1.86	NS	**↑↑	**↑↑
	SE ESTIMATE ± 0.95						
(iii) Proteins (mgs/gm wet liver)							
PRG	154.10 ± 5.50	163.10 ± 1.83	115.10 ± 2.73	153.20 ± 3.33	*↑	**↓↓	NS
CAT	161.20 ± 3.83	166.00 ± 3.10	119.46 ± 5.73	169.30 ± 2.56	NS	**↓↓	NS
GSH	159.56 ± 2.40	170.00 ± 2.33	122.66 ± 3.30	167.76 ± 3.07	*↑	**↓↓	NS
DF	160.23 ± 2.73	162.66 ± 1.95	119.90 ± 2.87	124.23 ± 3.37	NS	**↓↓	**↓↓
	SE ESTIMATE ± 2.79						
(iv) Xanthine oxidase (μmoles of xanthine oxidized/gm wet liver)							
PRG	12.67 ± 1.00	9.67 ± 0.60	24.66 ± 1.30	16.10 ± 0.67	*↓	**↑↑	*↑
CAT	15.87 ± 1.00	13.23 ± 1.20	26.87 ± 1.17	17.23 ± 1.07	*↓	**↑↑	NS
GSH	13.89 ± 1.20	11.33 ± 1.10	27.00 ± 2.03	15.33 ± 1.10	*↓	**↑↑	NS
DF	15.67 ± 1.30	13.43 ± 1.17	26.89 ± 2.17	26.46 ± 1.17	*↓	**↑↑	**↑↑
	SE ESTIMATE ± 0.81						

(v) Parasitaemia and survival/mortality

Antioxidants	Groups	Parasitaemia		Survival/Mortality mean	
		Px %	SEM	Xn	% Survival
PRG	IT	15.10	± 1.57	6	60
	IU	17.21	± 4.19	3	30
CAT	IT	17.32	± 2.56	7	70
	IU	22.97	± 2.89	3	30
GSH	IT	17.14	± 2.00	6	60
	IU	22.47	± 2.79	3	30
DF	IT	18.66	± 2.47	4	40
	IU	20.50	± 2.38	3	30

Key for Abbreviations: ↑ Rise in levels; ↓ Fall in levels; UU = Uninfected untreated; UT = Uninfected treated; IU = Infected untreated; IT = Infected treated; ($P < .01$) ** Highly significant; ($P < .05$) * Significant; ($P > .05$) NS Not significant; Px% = Mean per cent parasitaemia; SEM = Standard error of mean % parasitaemia; Xn = Number of animals surviving at the end of 28 days experiment, which is the mean of three sets of experiments.

Swiss mice (CDRI colony) and *Plasmodium berghei* (Vinckei and Lips strain, 1948) obtained from NICD, Delhi were employed in these studies.

One drug was studied at a time, employing four groups of mice (ten animals per group) which were distributed as follows:— normal, AO treated, infected and infected AO treated groups. The antioxidant was administered once in twenty four hours at a dosage of 15 mg/kg body weight subcutaneously. Of the two groups which were to be challenged with one million parasitized cells/mouse, one was treated with AO one hour after the challenge; the second infected group was not given any treatment.

The animals were sacrificed when the parasitaemia in control had reached upto 17-22% in about four weeks. The livers were collected, washed in normal saline, preserved in a deep freeze and tested for various biochemical parameters within 7-10 days (Halliuuell and Gutteridge, 1985; Litwack *et al.*, 1953; Utley *et al.*, 1967; Lowry *et al.*,1951). Experimental data on low range infection was not considered essential in the present study, since in our earlier study we had unambiguously shown that biochemical measures adopted against stress organ injury in plasmodial infection were equally effective both against initial low parasitaemia or higher/advanced stages of infection.

Thus logically counteraction of the stress organ injury at higher parasitaemia (longer infection) should be taken as a much more stringent criterion for establishing the efficacy of counteracting measures against this infection.

Recording of parasitaemia was done on alternate days. The three remaining antioxidants were studied in the same manner and the complete study on four antioxidants comprised one set of experiments. Two more similar sets of experiments were conducted on the four antioxidants and the data of the three sets of experiments was subjected to analysis of variance for one physical and the three biochemical parameters and has been summed up in Table 1.

RESULTS

It may be seen that *Plasmodium* infection caused highly significant increase in the liver weight of the infected animals, Table 1, 1(UU*/IU; $P<0.01$). The four antioxidants (AOs) themselves did not exert any statistically significant/noticeable effect on the stress organ (liver) of the uninfected animals, Table 1, 1 (UU*/UT; $P>0.05$). However, treatment of the infection (i.e., of the infected animals) simultaneously with GSH completely neutralized the infection effect of increase in liver weight, Table 1, 1(UU/IT; $P>0.05$), the difference between UU and IT vis-a-vis the liver weight being statistically insignificant. This means that GSH treatment could practically neutralize the infection effect of increase in liver weight, Table 1, 1(UU/IU vis-a-vis UU/IT). The neutralization of the infection effect was, however, only partial under the action of the two AOs— PRG and CAT, Table 1, 1(UU/IT; $P<0.05$). In these cases the difference in liver weight between UU and IT could only be incompletely bridged by the two AOs Table 1,1(UU/IU vis-a-vis UU/IT). The fourth AO, DPF practically failed to counteract the infection effect of increase in liver weight, Table 1, 1(UU/IT; $P < 0.01$). In other words DPF could not bridge the difference in liver weight between IT and UU, Table 1, 1(UU/IU vis-a-vis UU/IT).

The treatment of animals with four AOs, each administered individually did not affect the liver

The abbreviations, IU, UU, IT and UT imply infected untreated, uninfected untreated, infected treated and uninfected treated groups respectively.

* Summary of the data of three sets of experiments on biochemical parameters under the action of the four antioxidants depict analysis of variance and results of significance.

lipid peroxide levels LLP; Table 1 , 2(UU/UT; $P > 0.05$). *Plasmodium* infection significantly increased the LLP levels, Table 1, 2(UU/IU; $P < 0.01$). Treatment of the infected animal with each of the three AOs, PRG, CAT and GSH brought down the level of this biochemical parameter to an extent that the difference of biochemical alterations was statistically insignificant between the infected group and another infected group which was simultaneously treated with the AO, Table 1, 2(UU/IT; $P > 0.05$), which means that the three AOs could practically neautralize this adverse effect of infection i.e., increased levels of liver lipid peroxides, Table 1, 2(UU/IU vis-a-vis UU/IT).

The fourth AO, DPF failed to counteract the infection effect as monitored by the LLP levels in the stress organ, Table 1, 2(UU/IT; $P < 0.01$) which means that LLP levels remained high despite DPF treatment, implying that compound DPF could not bridge the difference between UU and IT as to the LLP levels, Table 1, 2(UU/IU vis-a-vis UU/IT).

Of the four AOs, two—PRG and GSH were found to increase the liver protein levels, LPL in the uninfected and animals, Table 1, 3(UU/UT $P < 0.05$). The infection alone brought about a highly significant fall in LPL levels, Table 1, 3 (UU/IU; $P < 0.01$). Simultaneous treatment of infected animals with an AO exerted the wholesome effect of counteracting this LPL disturbance by raising the liver protein levels under the action of three AOs, PRG, CAT, GSH, Table 1, 3(UU/IT; $P < 0.05$), practically bridging the difference between UU and IT for the liver protein levels, Table 1, 3(UU/IU vis-a-vis UU/IT).

The fourth AO, i.e., DPF failed to counteract the adverse effect of infection by failing to raise and thereby correct the liver protein fall as judged by the above criteria.

All the four AOs, PRG, CAT, GSH and DPF displayed the property of lowering the liver xanthine oxidase (XO) levels in the uninfected animals, Table 1, 4(UU/UT; $P < 0.05$). The infection alone caused a highly significant increase in XO levels of infected animals, Table 1, 4(UU/IU; $P < 0.01$). Treatment with two AOs i.e., CAT and GSH could significantly nullify the adverse effects of infection, Table 1, 4 (UU/IU vis-a-vis UU/IT; $P > 0.05$ with respect to UU/IT), by lowering the liver XO levels, while PRG was only partially effective in lowering the raised levels of XO, Table 1, 4 (UU/IU vis-a-vis UU/IT; $P < 0.05$ as to the UU/IT). The AO, DPF was found to be totally ineffective in this respect, Table 1, 4(UU/IU vis-a-vis UU/IT; $P < 0.01$ in regard to UU/IT), it totally failed to bridge the gap between UU and IT.

In brief, while GSH could afford protection to the host stress organ against each of the above studied ill-effects of infection brought about by *Plasmodium berghei* in the host stress organs, DPF completely failed in this respect.

Catechin could give protection in case of three parameters while protection by it was partial in case of the fourth parameter of liver weight.

Propylgallate was partiallly effective in case of two parameters, XO and liver weight but it proved fully protective against lipid peroxidation and protein imbalance.

DISCUSSION

Though the antioxidants could not influence the parasitaemia to any significant extent (Table 1, v), the results of the study on the above lines fully justify the guiding premise that the antioxidants can afford protection to the host against injury incurred by its stress organs in *Plasmodium* infection presumably caused by harmful free radicals (Sharma *et al.*, 1978b; Pryor, 1976; Ciba Geigy Foundation, 1979; Singh *et al.*, 1987; Wozencraft *et al.*, 1985). Accordingly, it has been shown that the three antioxidants could actually give protection to the host

liver to different extents against the physical/ biochemical alterations caused by the infection (Sharma *et al.*, 1978b; 1978a; 1979). This points to the capabilities as well as to the limitations of the antioxidants in counteracting stress organ injury in *Plasmodium* infection. The variegated response invoked by the antioxidants against stress organ injury indicates the multifaceted and complicated nature of factors, which engender free radicals in the host, (the probable causative agents) of the above damage in *Plasmodium* infection (Sharma *et al.*, 1978b; Ciba Geigy Foundation, 1979; Wozencraft *et al.*, 1985). The involvement of free radicals as causative agents of stress organ injury in other hosts in this infection remains to be understood. Systematic studies on the above may prove fruitful and informative for a better understanding of the stress organ injury in malarial infection in general.

The overall success of the premise motivating the present study would encourage one into suggesting the conjoint use of innocuous but effective antioxidants so as to cover a maximum range of free radicals (Pryor, 1976 ; Clark *et al.*, 1986; Huang *et al.*, 1987; Steven *et al.*, 1988) for effectively guarding against the latter in malaria infection.

This is likely to afford a better understanding of *Plasmodium* infection at the molecular level. Besides, the present series of studies would enable one to look at *Plasmodium* infection at the molecular level. This approach coupled with antimalarial chemotherapy has put forth a new concept not hitherto contemplated for treating malaria for a speedier and a qualitatively better recovery not only from the view point of parasitaemia but also from that of organic injuries caused by the latter; a facet of malaria infection and its treatment practically ignored by researchers in this area. Efforts are also being made to develop non-invasive biochemical parameters as tools for monitoring stress organ injury as well as recovery of the host in *Plasmodium* infection under the action of combination treatment as contemplated above.

This is the fourth successful approach for counteracting the stress organ injury in *Plasmodium* infection; the earlier ones successfully employed as protectors of the stress organ in malaria infection, were N-acetyl Penicillamin, desferrioxamine and zinc oxide. This study like the previous ones of its genre (Singh *et al.*, 1985; Singh *et al.*, 1987; Arif *et al.*, 1987) opens up a new area in malaria research and treatment.

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Insecticide Impregnated Ropes as Mosquito Repellent

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Ropes were impregnated with different dosages of deltamethrin and burnt throughout the night in human dwellings and cattlesheds. Smoke from smouldering ropes treated with various dose levels gradually saturated rooms and prevented the entry of mosquitoes. This method provided very good protection from mosquito bites including the principal vector of malaria, *A. culicifacies*. Results of ropes impregnated with 80 ppm deltamethrin were more consistent than at the lower dosage. The technique is indigenous, cost-effective, simple and appropriate for rural areas and does not require any special skills in its application.

INTRODUCTION

In rural and urban areas of the country mosquito nuisance is unbearable, and in large parts of the country transmission of vector-borne diseases such as malaria, filaria, Japanese encephalitis and dengue fever is commonplace. Although spraying of residual insecticides and larviciding are the most common methods of mosquito control, personal protection methods have their place in reducing man mosquito contact. As a personal protection measure, coils containing synthetic pyrethroids and other insecticides have been used almost throughout the world with good results (Charlwood and Jolley, 1984), but the use of sound producing devices has not proved fruitful (Curtis, 1986). In India, a variety of devices such as coils, mats, electrical repellents etc. are sold in the market. These devices are expensive and some of them require electricity. For the large rural population particularly to those living in inaccessible areas and belonging to low socio-economic strata, there was no simple and cheap method to provide protection from mosquito bites. Therefore, there was a feltneed of a system that would be low cost, indigenous and readily available in even the most remote and backward areas of the country and would not require any special skills in its application or use. One such method was the use of ropes treated with synthetic pyrethroids which were allowed to smoulder to produce smoke toxic/repellent to mosquitoes and other haematophagous insects. Results of experiments with deltamethrin impregnated ropes are described in this paper.

MATERIAL AND METHODS

Ropes of different diameters and fibres were collected from the market. There is no uniform standard of ropes and they are made of jute (*patsan*) and coconut fibres etc.

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Table 1. Details of impregnation and burning of ropes

S. No.	Type of rope	Diameter in cm	Circum-ference in cm	Wt in gms per meter	Water absorbed per gm rope	Water absorbed per meter	Rope burnt in 1 hr in cm
1.	Sutli	0.8	2.5	23.6	1.36 ml	31.42 ml	12.5
2.	Coconut	0.875	2.75	23.03	1.24 ml	28.82 ml	11.76*
3.	Coconut	1.75	5.5	77.25	0.89 ml	69.09 ml	6.00*
4.	Jute	1.1	3.5	100.00	0.688 ml	68.90 ml	6.18
5.	Jute	1.75	5.5	115.00	0.92 ml	106.38 ml	4.35
6.	Jute	0.9	3.0	28.40	1.35 ml	38.46 ml	10.00
7.	Mixed fibres (Jute + Coconut)	1.1	3.5	72.40	0.47 ml	34.00 ml	5.00*

* Extinguished several times.

Table 2. Percentage of mosquito species collected during whole night collection

Species	Total anophelines %	Total mosquitoes %
Anopheles culicifacies	22.19 (1569)	18.63 (1569)
A. stephensi	0.32 (23)	0.27 (23)
A. annularis	2.48 (176)	2.09 (176)
A. subpictus	74.84 (5292)	62.85 (5292)
A. nigerrimus	0.15 (11)	0.13 (11)
A. pulcherrimus		
Total anophelines	100 (7071)	83.99 (7071)
Total culicines	—	16.01 (1348)
Total mosquitoes	—	100 (8419)

Figures in parentheses indicate the total number of mosquitoes collected.
Total 96 collections from 1900-0600 hrs in Aug-Sept 1989.

Deltamethrin 2.5% flowable concentrate was used to impregnate the ropes. Initially ropes were impregnated by soaking in insecticide suspension of different concentrations for about 1-2 hours, and allowed to dry in shade. However, it was problematical to obtain consistent quality of impregnation with different type and thickness and different water absorption capacity. In order to obtain a uniform concentration and to standardise the procedure, ropes were selected on the basis of water absorption capacity and uninterrupted burning during the whole night. Water absorption was calibrated by measuring the volume of water after soaking ropes of known weight in a known volume of water. Based on absorption capacity and uninterrupted burning during the whole night, Jute (*patsan*) ropes (0.9 cm dia) were selected for further trials. These ropes were impregnated with 1, 5, 10, 20, 40 and 80 ppm i.e., (mg/kg wt. of the rope). Deltamethrin flow (2.5%) water suspension was supplied by the courtesy of M/s. Roussel Pharmaceuticals India Ltd.

Deltamethrin is produced in India. It is marketed for agriculture under the trade name of Decis (E.C. formulation) and as K-othrine (W.P. formulation) for use in public health. It is a colourless crystalline white powder, soluble in acetone, ethanol, dioxan and most aromatic solvents but practically insoluble in water (< 0.002 ppm). It is stable on storage and no degradation has been reported even after 6 months at 40°C (WHO, 1984).

The ropes were hung in rooms of village houses and cattlesheds, burnt from one end and allowed to smoulder through the night. Smoke from the smouldering rope containing deltamethrin gradually permeated the air.

Field studies were carried out from August to September 1989 during the malaria season which also happens to produce high mosquito densities. Experiments were carried out in Pipalhera, Kichera and Dehra villages of Dhaulana PHC and Ramgarh village of Dadri PHC, District Ghaziabad, U.P. For each concentration of treated rope a control was held without rope and with plain rope (without impregnation). A total of 8 all night collections were made in houses (rooms) and 8 night collections in cattlesheds. Mosquito collection was made for 15 minutes in each room or cattleshed at hourly intervals from 1900 hrs to 0600 hrs. Total mosquitoes collected were identified, recorded and averaged for one night. Results of the study were calculated on the basis of protection provided against collection from rooms or cattlesheds held without ropes.

RESULTS AND DISCUSSION

Table 1 provides details of ropes obtained from the market for insecticide impregnation. In preliminary experiments it was revealed that coconut ropes have low water absorption capacity and they stopped smouldering several times during the night. Even ropes made of jute and coconut extinguished while smouldering. Jute ropes were found suitable as their water absorption capacity was better and thus insecticide impregnation was uniform and they smouldered all night. It was possible to obtain uniform impregnation of ropes whether treated by weight or by dipping in a known concentration of deltamethrin in water. Jute ropes were dipped in various water concentrations of deltamethrin for field experiments and this method was found simple and produced uniformly treated ropes.

Table 2 gives data of the total number of mosquitoes collected in rooms and cattlesheds calculated to show the abundance of different mosquito species. Results revealed that in this area *Anopheles culicifacies* is the vector species and other anophelines comprise mainly of *A. subpictus* with small numbers of *A. annularis, A. stephensi, A. nigerrimus* and *A. pulcherrimus. Culex quinquefasciatus* is the most dominant culicine mosquito. Among anophelines *A. subpictus* populations comprised about 75% and *A.*

Table 3. Results of deltamethrin impregnated ropes

Dosage of delta-methrin (PPM)	*A. culicifacies*			Total anophelines			Total culicines			All mosquitoes		
	Expt.	Cont.	% protection	Expt.	Cont.	% protection	Expt.	Cont.	% protection	Expt.	Cont.	% protection
Human dwellings (Rooms)												
0	66.5	87.5	24.3 ± 6	161.0	210.2	24.8 ± 11.9	20.2	33.2	44.1 ± 17.4	181.2	243.5	28 ± 12.1
1	40.7	87.5	47.5 ± 4.7	89.1	210.2	54.4 ± 6.2	18.8	33.2	38.3 ± 23.8	108.0	243.5	47 ± 11.7
5	32.7	87.5	58.8 ± 3.4	89.5	210.2	54.7 ± 5.3	17.7	33.2	47.0 ± 11.7	107.2	243.5	54 ± 6.2
10	1.7	5	55.8 ± 5.1	57.0	136.3	39.8 ± 26.9	4.0	9	41.1 ± 34.0	61.0	145.3	46.2 ± 15.6
20	4.8	12.2	62.8 ± 23.5	72.0	163.3	56.3 ± 20.7	11.1	29.1	39.9 ± 31.2	83.1	192.5	55.0 ± 21.0
40	1	12.2	95.1 ± 12.5	37.3	163.3	77.6 ± 9.6	8.0	29.1	59.4 ± 36.3	45.3	192.5	76.4 ± 8.1
80	1	16.8	93.5 ± 5.7	24.2	142.6	83.3 ± 5.9	20.0	45.8	58.4 ± 20.3	44.2	188.5	78.3 ± 0.5
Cattlesheds												
0	89.2	125.5	30.0 ± 9.1	206.5	313.2	31.7 ± 18.0	37.2	52.0	27.6 ± 16.2	243.7	365.2	31.1 ± 17.5
1	52.6	125.5	51.8 ± 14.9	128.1	313.2	52.0 ± 16.4	20.2	52.0	56.0 ± 20.0	148.3	365.2	52.3 ± 16.3
5	47.5	125.5	58.4 ± 9.6	117.5	313.2	56.8 ± 15.1	22.1	52.0	54.1 ± 15.6	139.6	365.2	56.3 ± 15.2
10	3.25	16.6	67.9 ± 20	89.9	363.0	51.5 ± 36.9	12.0	32.8	54.3 ± 7.9	101.8	396.5	55.5 ± 40.9
20	4.3	20.2	77.1 ± 13.3	65.2	366.0	75.9 ± 7.1	11.2	29.1	64.1 ± 21.0	80.2	395.1	74.0 ± 7.4
40	3.8	20.2	83.4 ± 12.7	160.1	366.0	78.0 ± 5.5	10.0	29.1	82.0 ± 14.6	170.1	395.1	81.3 ± 5.5
80	2.3	17.0	85.7 ± 7.6	26.0	250.5	88.9 ± 0.6	13.6	31.7	74.9 ± 9.9	39.6	282.1	86.1 ± 2.1

Notes :
1. Density figure for mosquitoes are for whole night collection (1900-0600 hrs) averaged for one night.
2. One set of control was held for various experimental ropes and therefore control data has been repeated in the table at various dose levels.
3. Protection calculated by subtracting total mosquitoes in expt. room from the control and divided by control and multiplied by 100.
4. Great variation in mosquito density was observed from village to village and from night to night collection.
5. The experiments were carried out from Aug.-Sept. 1989 and generally less mosquitoes were found towards the end of September.

culicifacies about 22%; the remaining 3% comprised mainly of *A. annularis* and all other were represented by a few numbers. If all mosquitoes are counted then *A. subpictus* is most abundant accounting for about 63% population whereas *A. culicifacies* and *Cx. quinquefasciatus* constituted about 19 and 16% population and all other species were found in insignificant numbers, except *A. annularis* which comprised about 2% of the population.

Table 3 gives the results of mosquito collection in human dwellings (rooms) and cattlesheds. Results shown are the average per night collection calculated from 16 night (1900 hrs to 0600 hrs) collections. The protection provided by the smouldering ropes increased with the increasing concentration of deltamethrin. Good protection was provided at concentrations from 10, 20 and 40 ppm with high variation in the replicates. In rooms, protection from *A. culicifacies* and the vector of malaria in this area varied from 55 to 95% at 10, 20 and 40 ppm but there was high variation among the replicates. At 80 ppm, variation among the replicates was greatly reduced and the results were stable. Although protection from all other anophelines and culicines was relatively less as compared to *A. culicifacies* and much less for *Culex quinquefasciatus* but the results were more consistent and high degree of protection from mosquitoes was provided in the rooms. A similar phenomenon was found in the cattlesheds although with relatively reduced effectiveness. Most mosquito species whether vector or non-vector are basically zoophilic and zoophagic and therefore this reduced effectiveness in the cattlesheds was expected.

An interesting feature of all night collection of mosquitoes was their sudden influx in rooms and cattlesheds at dawn (0500 hrs to 0600 hrs). Table 4 gives the results of entry of mosquitoes in houses and cattlesheds with burning ropes treated at various dose levels. The data is pooled together for all night and one hour collection between 0500 to 0600 hrs. Results revealed that about 13-22% of the total *A. culicifacies* entered rooms between 0500-0600 hrs in August-September 1989. A large population of *A. subpictus* (20-40%) entered rooms during dawn. In case of *Cx. quinquefasciatus* about 10% enter the rooms between 0500-0600 hrs which is the normal rate of entry at other hours in the night. Mosquito entry in the morning hours is mainly for resting and not for biting. Since the urge to find a suitable resting place is strong, a large number of mosquitoes enter houses. The protection provided against mosquitoes as computed in Table 3 includes the influx of mosquito populations during the dawn period. If this population which enters houses and cattlesheds for resting during the day time is deleted from calculations on protection from impregnated ropes then the effectiveness of ropes will increase substantially. Deltamethrin impregnated ropes therefore provide high degree of protection from mosquito nuisance and bites. Based on field studies a dose of 80 ppm may be appropriate for use and at this dosage man mosquito contact of *A. culicifacies* would also be reduced by >90%. Such a major reduction in *A. culicifacies* densities may have desirable impact on the reduction in malaria transmission.

Mosquito coils manufactured in India may use natural pyrethrum or synthetic pyrethroids and a suitable cellulose material containing inorganic nitrates to regulate burning. The exact amount of insecticide and its formulation and/or mixture of insecticide is not mentioned and is held as a trade secret. The commercial coils from East Africa and Hong Kong contain 0.19—0.31% pyrethrins. Chinese coils contain 7.4—13% DDT (Hudson and Esozed, 1971) and coils in Kenya contained 0.044% and 0.099% Esbiothrin (Birley *et al.*, 1987). DDT coils were not found to produce any repellent action but synthetic pyrethroids produced good repellency (Chadwick, 1975). Chemical analysis of Indian coils showed that these are safe to human health (Saini *et al.*, 1986). The concentration of deltamethrin used in the ropes (upto 80 ppm) was

Table 4. Number of mosquitoes entering dwellings with smouldering deltamethrin treated ropes at various dose levels

Species	Pooled data of 64 replicates 11-30 Aug. 1989			Pooled data of 32 replicates 6-13 Sept. 1989			Pooled data of 96 replicates 11 Aug-13 Sept. 1989		
	1900-0600 hrs.	0500-0600 hrs.	%	1900-0600 hrs.	0500-0600 hrs.	%	1900-0600 hrs.	0500-0600 hrs.	%
A. culicifacies	180	40	(22.22)	1389	174	(12.52)	1569	214	13.63
A. subpictus	3331	1398	(41.96)	1961	174	(20.24)	5292	1796	33.93
Total anophelines*	3677	1417	(38.53)	3394	591	(17.41)	7071	2008	28.39
Culicines	718	73	(10.16)	630	79	(12.53)	1348	152	11.27
Total mosquitoes	4395	1490	(33.90)	4240	670	(16.65)	8419	2160	25.65

* Also includes *A. stephensi, A. annularis, A. nigerrimus* and *A. pulcherrimus* which were encountered in small numbers.

very low compared to the recommended concentration of other pyrethroids. Use of deltamethrin impregnated ropes will therefore be useful in the prevention of man mosquito contact, instead of the use of mats or coils which are expensive and may require electric supply.

Mosquito bednets are also used to prevent mosquito bites and transmission of malaria. In recent years insecticide impregnated bednets have proved effective in the control of malaria (Lindsay and Gibson, 1988). A recent study in Sonapur (Assam, India) has shown that there was reduction in malaria transmission in populations sleeping under deltamethrin impregnated bednets. The areas are under the influence of *A. minimus*, *A. balabacensis* (= *dirus*) and *A. philippinensis* (= *nivipes*) and spraying of DDT was not producing any tangible impact on transmission (Vas Dev, personal communication). Mosquito nets are expensive and invariably there is some resistance to their routine use. Smoke from coils or ropes provides better protection since the rope can be burnt from dusk to dawn whereas most people use bednets after dinner time. Besides the ropes provide protection to the entire family in a room. Ropes are made in villages all over the country, from jute or other material. The rope burns slowly for about 10-12 hours and the resultant smoke has the desired repellent and at times killing effect on mosquitoes.

Field experiments have clearly demonstrated that deltamethrin impregnated ropes are effective in repelling mosquitoes in villages with open doors and windows. The dosage of 80 ppm provides good protection and ropes of lower dosages could be used in closed rooms. However if the rooms are closed effectiveness of ropes would increase considerably. In one night about 1.2 metres of rope burns which costs about 50 paise (US $ 0.03). The technique should also be useful during peak transmission season or in areas with JE epidemics or where only occasionally high mosquito populations build up or where local people have resistance to the use of bednets. Field experiments are in progress to study the repellent action of impregnated ropes on various mosquito vector species and other haematophagous insects.

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Seroepidemiological Study of Malaria in a Rural Population of Chandigarh

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A total of 1689 peripheral blood smears and serum samples were collected from healthy subjects from four villages of U.T. Chandigarh during the pre-monsoon season (February to May 1987) while 1809 such samples were collected during the post-monsoon season (October 1987–January 1988). None of the peripheral blood smears examined by Giemsa and acridine-orange stains showed malarial parasite. Out of 1689 serum samples tested by indirect haemagglutination (IHA) test during pre-monsoon season, 81 per cent showed malarial antibody titres of less than 1:8 and only 19 per cent showed titres of 1:8 or above. In contrast, out of 1809 serum samples tested during post-monsoon season, 58.3 per cent showed antibody titres of less than 1:8 while 41.6 per cent samples showed titres of 1:8 and above. Total number of malaria cases from these villages from June 1987 to January 1988 was also low (total 65 cases) as compared to corresponding period of previous year (total cases 191). Serological findings independent of positive cases of malaria suggest that though, no proved clinical cases of malaria were observed in the population surveyed, malaria transmission had certainly taken place as evidenced by higher antibody titres observed during the post-monsoon season compared to pre-monsoon season.

INTRODUCTION

After resurgence of malaria in the seventies due to well-known reasons (Sharma and Mehrotra, 1986) the conventional indices used for measurement of endemicity like spleen rate and parasite indices were found to be insensitive for determination of the precise malaria status (Ray and Beljaev, 1984). The immune status of the host and indiscriminate use of antimalarial drugs for treatment and chemoprophylaxis also made epidemiological indices dependent on malarial parasite detection almost invalid (Kagan, 1972; Sadun, 1972). To overcome this problem, many workers have used different serological techniques for the study of epidemiology of malaria. In the present study indirect haemagglutination (IHA) test was chosen because of its simplicity in performance, suitability for field conditions and no requirement of any special sophisticated equipment. (Mathews *et al.*,1975; Kagan, 1972; Meuwissen, 1974; Chandanani *et al.*, 1981). Further, as *Plasmodium vivax* and *Plasmodium falciparum* antigens are not easily available in most laboratories, simian plasmodia have been employed as the source of antigens. *P. knowlesi*

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Table 1. Agewise distribution of persons showing antimalarial antibody titres of ≥ 1:8

Age group (yrs)	Pre-monsoon survey					Post-monsoon survey				
	Kaimbwala	Daria	Railway Colony	Bapudham	Total	Kaimbwala	Daria	Railway Colony	Bapudham	Total
< 5	17/216	14/116	13/88	17/186	61/606	32/82*	42/116*	14/25*	61/140*	149/363*
5-14	29/217	18/68	8/34	16/105	71/424	75/182*	26/97	59/100*	36/103*	196/482*
15-45	37/152	71/218	29/115	40/150	177/635	105/270**	85/253	109/186*	77/173*	376/882*
> 45	5/12	2/4	0/3	3/5	10/24	14/34	5/22	3/9	10/17	32/82
Total	88/597	105/406	50/240	76/446	319/1689	226/568**	158/488***	185/320*	184/433*	753/1809*

*P value < 0.001; **P value < 0.01; ***P value < 0.05.

antigen has been found satisfactory for diagnosis of human malaria (Chandanani *et al.*, 1981; Srivastava *et al.*, 1983). Not much information is available from our country regarding the value of serology in determining the epidemiology of malaria. With these points in view, we have been conducting studies to assess the seropositivity and blood smear positivity in the rural population of Union Territory of Chandigarh during the last few years. During the year 1987, the incidence of malaria in Chandigarh area had markedly decreased, probably due to scanty rains resulting in widespread drought in the country during that period. We report here an interesting epidemiological observation of malaria transmission made in rural population of Chandigarh during that period.

MATERIAL AND METHODS

Four villages of Union Territory of Chandigarh, namely Kaimbwala, Daria, Railway Colony and Bapudham Colony were selected for the study. Two surveys, one pre-monsoon and the other post-monsoon were carried out in these areas. In both the surveys, only normal healthy individuals not having fever at the time of surveys were included, persons having fever were also investigated but were recorded separately. For recording personal data, 'Individual case proforma' of Indian Council of Medical Research (ICMR) was used and accordingly the individuals were divided in four age groups viz., <5 yrs, 5-14 yrs, 15-45 yrs and above 45 yrs. From each individual two blood smears and serum samples by finger prick were collected on two Whatman No. 3 filter paper discs of diameter 10 mm (WHO Memorandum, 1974). The smears were fixed in methanol. One smear was stained with Giemsa and the other with acridine orange (Shute and Sodeman, 1973). Antimalarial antibodies were detected by indirect haemagglutination (IHA) test according to the method described by Meuwissen (1974) using *P. knowlesi* antigen. During the pre-monsoon surveys (February 1987 to May 1987) a total of 1689 samples were collected from four villages which covered 26 per cent of the population. During the post-monsoon survey (October 1987 to January 1988) a total of 1809 samples were collected covering 28 per cent of the population.

RESULTS

None of the blood smears stained with Giemsa or acridine orange stains was positive for parasites from either the pre-monsoon and post-monsoon surveys. Table 1 shows the antimalarial antibody titres in the serum samples tested from the four villages during these surveys. In the table only the individuals who were not having fever at the time of survey were included. In all the age groups except the group above 45 yrs a higher number of persons showed antimalarial antibody titres of 1:8 or above in the post-monsoon survey than in the pre-monsoon survey in all the four study areas except in Daria where only <5 yrs age group showed significant difference. Similarly, considering the various age groups as a whole from all the four villages, in all the age groups except that of above 45 yrs, a significantly higher number of persons showed antibody titres of 1:8 or above during the post-monsoon season (Table 1). When all the age groups were combined, in pre-monsoon survey 81 per cent samples from these villages showed antibody titre of less than 1:8 and only 19 per cent showed antibody titres of 1:8 or above. During the post-monsoon survey, out of 1809 samples tested 58.3 per cent showed antibody titre of less than 1:8 while, 41.7 per cent of serum samples had antibody titres of 1:8 or above, which was significantly higher as compared to only 19 per cent of serum samples having titres of 1:8 or above during pre-monsoon survey ($p < 0.001$).

Among the individuals having fever, the number of persons positive for malaria parasite in blood smear during monsoon and post-monsoon period (July 1987—January 1988) in these villages was: Kaimbwala-11, Daria-21, Railway Colony-6 and part of Bapudham Colony-27. Majority of the cases with malaria infection showed signifi-

cant levels of antimalarial antibodies (data not shown).

DISCUSSION

As is evident from Table 1, higher number of persons from all the four villages showed significant levels of antimalarial antibodies during the post-monsoon period as compared to pre-monsoon period. This data, particularly in lower age group of <5 yrs, indicates that though we did not have clinical cases of malaria among the persons surveyed, transmission of malaria had certainly taken place during the monsoon and post-monsoon period as evidenced by the higher antibody levels in the population. Similar observations had been made by Draper *et al.* (1972) when they compared the serological profile and blood smear positivity between three different areas with different levels of malaria transmission in Africa. The individuals showing significant levels of antibody must have had sub-clinical infection with low (subpatent) levels of parasitaemia. This observation was substantiated by the fact that though, there was no clinical case of malaria in the population surveyed, there were cases of malaria occurring in the population as detected by blood smear examination of individuals having pyrexia. The total number of malaria cases detected in these villages from July 1987 to January 1988 was 65. The incidence of positive cases was lower than that observed in previous year (1986) which was 34, 84, 21 and 52 cases in village Kaimbwala, Daria, Railway Colony and Bapudham Colony respectively for the corresponding period, indicating thereby that in these areas the transmission of malaria was lower than in the previous year.

Thus serological survey could certainly play an important part in determining the epidemiology of malaria, particularly in reflecting the transmission period or different levels of transmission in various seasonal or climatic conditions.

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Mosquito Breeding in Rice Fields and its Role in Malaria Transmission in Mandla district, M.P.

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A study of the breeding of mosquitoes in the rice field agro-ecosystem was carried out in Mandla district during 1987 and 1988. Results revealed that mosquito breeding commences in July and ends in October. Mosquito breeding in rice fields is inversely proportional to the distance from village. *A. culicifacies* breeds in rice fields upto a plant height of 20 cm and then gradually declines and becomes scarce, and is taken over by *A. theobaldi* and *A. splendidus*. *A. fluviatilis* breeds in irrigation channels almost throughout the rice growing season. There were a large number of other mosquito breeding sites which increased enormously with the onset of rains. The precise role of rice fields in maintaining high malaria transmission could not be established but the rice field agro-ecosystem contributed significant vector populations.

INTRODUCTION

Rice is a staple food in India and it is grown extensively in all parts of the country. In Madhya Pradesh (M.P.) 5 million hectares are under rice, which is approximately 12% of the total acreage under rice in the country. The annual rice production is about 3800 tons and contribution of M.P. towards total rice production in India works out to 6.4%.

In Mandla, rice is the main crop grown in small holdings. Agriculture is primitive, monsoon dependent and use of modern agricultural practices is minimal. Mosquito breeding is commonly encountered in the standing water in fallow fields, rice fields, rainwater collections and water stored for irrigation.

Bizadandi block is endemic for malaria with high degree of transmission and evidence of resistance to chloroquine and metakelfin (Singh *et al.*, 1989a). At present very little is known about the role of vast areas under rice cultivation on vector abundance with particular reference to malaria. A study was therefore, taken up to investigate mosquito breeding in rice fields with the aim to study the breeding of *A. culicifacies* in rice fields, ecological succession of mosquitoes in the rice field ecosystem and the relationship of rice cultivation to malaria. Results of this study are reported in this paper.

MATERIAL AND METHODS

This study was conducted in Bizadandi block. In this area malaria control under the Integrated

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Disease Vector Control (IDVC) project is in progress since May 1986, using the biological control and environmental management methods and no insecticides have been applied for malaria control (Singh *et al.*, 1989b). Bizadandi block covers a total of 471 sq kms and has a population of 55,000 of which 80% are tribal (Gonds). Most people live in hamlets. Terrain on the whole is undulating with small, perennial rivulets and streams.

Climate of this area has extremes i.e., high temperature in the summer months (May–June: 40°–80°C) and low during winter (Dec–Jan: from 4°–10°C). The relative humidity is 60–95%. Rainfall of the district varies from 975–1440 mm and the height above sea level is 710 meters.

Villages selected for the study are all situated in the rice tract near streams and rocky hills. These are generally flooded during July–August. In this area generally one and rarely 2 crops of rice are grown. No insecticide or fertilizer is used. Prior to the commencement of monsoon (June) rice is sown in a nursery which has 5 to 15 cms standing water. Seedlings are transplanted in submerged fields. There are no irrigation facilities but water is collected in the fields by digging small wells. After the rains algal growth invariably covers the water surface. Many paddy fields are situated on either side of the streams so that crops are flooded with water which is allowed to stand continuously for months. Since irrigation is monsoon dependent, the fields remain dry for a few days creating innumerable water puddles.

Studies were undertaken in rice fields situated at the periphery of villages, at about 1/2 km and 1 km distance. Weekly sampling of the immatures was carried out in each rice field. For density measurement 30 experimental plots (10 each at the periphery, 1/2 km and 1 km distance) were selected before the rice transplantation and observations were made on weekly basis until the harvest. Larval collections were restricted to only 20 dips (250 ml dipper) from each plot and this procedure was maintained throughout the period of observation. Ten dips on the surface of algal and other vegetation and 10 in the areas free from vegetation were taken. The larvae and pupae collected were brought to the laboratory, counted instarwise and reared to adult stage in the laboratory for identification. Larval collections were also made from other breeding places (random), identified and recorded in a similar manner. The adults that emerged from these larvae were identified with the help of keys by Christophers (1933) and Barraud (1934).

Simultaneously, weekly parasitological surveys were carried out. The study areas were classified as group A comprising 10 low incidence villages (API, 136 and 167 for 1987 and 88 respectively) and group B comprising 12 high incidence villages (API, 285 and 231 for 1987 and 88 respectively). Both groups of villages, A and B had comparable socio-economic status and geographical factors.

Blood smears taken from infants (0–1 yr) and children (2–9 yrs) were examined during the rice (rainy season) and non-rice season (winter and spring). Since this study deals with the transmission period in the area, the details of observations carried out during 1987 and 1988 are presented below.

RESULTS AND DISCUSSION

Larval collections revealed the presence of 11 species of anophelines and three species of *Culex* i.e., *A. culicifacies, A. subpictus, A. nigerrimus, A. fluviatilis, A. theobaldi, A. jamesii, A. annularis, A. vagus, A. splendidus, A. jeyporiensis, A. stephensi* (very rarely) *C. quinquefasciatus, C. vishnui* and *C. tritaeniorhynchus*. Table 1 gives the larval density in rice fields. Average larval density based on two years' observations revealed that mosquito breeding was inversely proportional to the distance from the village i.e., it was maximum at the periphery and minimum at 1 km distance.

Table 1. Density of immatures in rice agro-ecosystem in Bizadandi block, Distt. Mandla

Breeding sites observed during 1987	Distance from village	Average larval density per dip						
		July (16-31)	Aug* (1-15)	Aug (16-31)	Sept (1-15)	Sept (16-30)	Oct (1-15)	Oct (16-31)
Rice field	Upto 0.5 km **	11.36	2.84	13.21	18.29	8.40	4.48	3.25
Rice field	0.5-1.0 km **	2.32	0.80	1.07	2.51	1.30	3.23	All dry
Rice field	1.0-1.5 km **	1.38	0.74	3.92	3.42	3.87	2.99	3.70
Permanent breeding sites (Ponds and Streams etc.)	Random	12.49	10.85	6.86	15.49	12.18	12.30	15.21
Temporary breeding sites (Ditches, Pits, Rainwater and Hoof-prints etc.)	Random	15.23	13.12	29.90	17.58	17.00	10.48	14.73

* Low density in August second fortnight was due to heavy rainfall (15 cm in a couple of days).

** 10 rice fields were fixed for sampling.

In most villages there are a number of borrow pits, ditches and other excavations and invariably there is a small tributary which is used for washing clothes by the inhabitants. All these sites are filled up during rainy season and support *A. culicifacies* breeding. Table 1 shows that the number of larvae found randomly in these breeding sites were more than that in the rice fields and this might be the reason for high vector populations throughout the year.

Tables 2 and 3 give data on the proportion of different anophelines and culicines recorded in relation to the plant growth and proximity of rice fields to the village. Results revealed that *A. culicifacies* and *A. subpictus* were most abundant in the early stages i.e., till the plants reached a height of 20 cms (Table 2). Generally, mosquito larvae are not found in regions devoid of vegetation/algae. However, heavy breeding was encountered in small shallow pools and pits with and without vegetation and these areas continued to breed until they dried up in October-November. During September-October as the water level receded in rice fields, algal cover surfaced and this habitat supported mosquito breeding. Extensive growth of aquatic algae on the water surface and grass at the edges of rice fields was observed with the declining trend in the breeding of *A. culicifacies* and succession of *A. theobaldi* and *A. splendidus*.

Peak anopheline larval density occurred one week after planting which was associated with algal growth. The density of breeding appears to be governed more by the height of paddy plant (Neogy and Kachroo, 1956) algal activity and the interspace between the paddy plants (Russel *et al.*, 1963). In Mandla, *A. culicifacies* densities decreased and the species became very scarce after rice plants reached a height of 40 cms.

The rice fields also supported a variety of other aquatic organisms i.e., fish and insects, especially in water-logged fields which are situated on either sides of the streams and tributaries. Even though *Rasbora* (a larvivorous fish) and predatory insects were present in the rice fields, this

Table 2. Relationship of plant height to different mosquito species found breeding in rice fields*

	Emergents	Height of plant (cm)						
		0	1-20	21-40	41-60	61-80	81-100	>100
Anopheline species								
1.	*A. culicifacies*	66.10	55.11	35.58	14.80	7.45	3.50	3.86
2.	*A. subpictus*	15.17	19.14	15.68	9.76	7.51	3.09	0.24
3.	*A. theobaldi*	1.52	0.88	5.52	12.61	8.24	3.84	9.87
4.	*A. splendidus*	—	0.19	3.37	4.48	8.34	19.71	42.21
5.	*A. vagus*	0.55	0.15	0.77	5.89	1.89	0.15	—
6.	*A. hyrcanus*	—	0.19	0.25	0.36	6.62	2.23	2.03
7.	*A. annularis*	—	0.21	1.18	0.48	0.95	0.23	—
8.	*A. fluviatilis*	0.85	0.38	0.06	—	1.81	0.69	0.68
9.	*A. jamesii*	—	—	0.49	0.99	0.29	1.81	—
10.	*A. stephensi*	0.08	—	—	—	—	—	—
11.	*A. jeyporiensis*	—	—	0.62	—	0.45	—	—
	Total Anophelines	84.32	76.27	63.52	49.39	43.56	35.27	58.88
	Total Culicines	15.68	23.73	36.48	50.61	56.44	64.73	41.12

* Average of three types of rice fields situated at periphery 1/2 km and 1 km away from the village (10 villages).

Table 3. Proportion of mosquitoes found breeding in rice fields at various distances from the village

Emergents		Distance of rice fields from village		
		0-0.5 km	0.5-1 km	1-1.5 km
Anopheline Species				
1.	*A. culicifacies*	28.93	54.43	20.49
2.	*A. subpictus*	26.87	4.76	6.10
3.	*A. splendidus*	2.96	6.07	12.14
4.	*A. theobaldi*	1.74	3.93	8.65
5.	*A. vagus*	2.02	—	1.49
6.	*A. hyrcanus*	0.21	2.62	1.06
7.	*A. annularis*	0.58	0.82	0.46
8.	*A. fluviatilis*	0.21	0.49	0.99
9.	*A. jamesii*	0.16	0.33	0.30
10.	*A. jeyporiensis*	0.26	0.16	—
11.	*A. stephensi*	0.05	—	—
	Total Anophelines	63.99	73.61	51.67
	Total Culicines	36.01	26.39	48.33

* Average of 10 rice fields.

habitat continued to be an important breeding source for mosquitoes as the algal cover shielded mosquito larvae from predation.

Anophelines were most abundant during July, later *Culex* species progressively increased but conditions for breeding again became favourable for anophelines after the monsoon, especially for *A. splendidus* and *A. theobaldi*. *A. fluviatilis* was also found breeding in rice fields along streams, irrespective of the height of plants and distance of field from village. It constituted about 0.56% of the total emergence (0.21–0.99; Table 3) in different stages of plant growth. Most rice fields in which *A. fluviatilis* breeding was found, had a perceptible flow of water. Rao (1984) assessed that rice fields, though ecologically not an ideal breeding habitat for malaria vectors, may play a very important role in the epidemiology of malaria by building up high adult densities because of extensive surface areas of rice fields in and around villages, especially during rainy season.

Tables 4 and 5 give the epidemiological data of two groups of villages each of which had low and high malaria incidence during 1987-88. Epidemiological data revealed that malaria over a two year period exhibited a definite seasonal trend. Cases in spring mainly comprised *P. vivax* and a peak was followed by the onset of monsoon and active transmission continued till August–Sep-

Table 4. Epidemiological situation of malaria in Group-A villages, Bizadandi block, Distt. Mandla (M.P.)

Month	1987						1988					
	Total BSE	Total +ve	Total Pf	Slide positive rate			Total BSE	Total +ve	Total Pf	Slide positive rate		
				Infant	Child	Adult				Infant	Child	Adult
Jan	137	3	0	—	5.26	1.04	131	20	11	—	10.71	16.67
Feb	106	3	—	—	3.70	2.70	122	19	5	—	25.00	13.33
Mar	103	2	—	—	—	2.82	168	23	4	—	12.24	14.41
Apr	289	23	5	8.33	3.92	8.85	284	51	9	—	16.19	20.12
May	368	39	3	—	6.17	12.55	619	144	7	8.69	18.39	26.07
Jun	367	55	3	14.28	7.95	17.36	425	48	5	—	7.76	12.78
Jul	428	64	9	5.00	10.91	17.11	347	96	14	—	19.30	55.42
Aug	392	99	14	10.00	22.35	26.60	592	123	38	—	15.68	22.81
Sep	307	108	18	—	35.16	36.19	637	139	82	—	16.98	23.83
Oct	820	191	79	—	23.68	23.41	271	75	56	16.67	24.07	28.91
Nov	197	53	39	28.57	33.33	25.17	157	43	32	—	24.24	28.46
Dec	199	38	34	—	34.48	17.07	139	52	36	—	30.77	42.11
Total	3713	678	204	6.48	16.95	19.10	3892	833	299	3.85	17.48	23.69
	Gametocyte rate : Infant = 4.63%; Child = 11.83%; Adult = 20.18%.						Gametocyte rate : Infant = 2.56%; Child = 8.44%; Adult = 25.66%.					
	Pop - 5,000; SPR - 18.26; ABER - 74.26; API - 135.60; Pf% - 30.9.						SPR - 21.40; ABER - 77.84; API - 166.60; Pf% - 35.89.					

Table 5. Epidemiological situation of malaria in Group-B villages, Bizadandi block, Distt. Mandla (M.P.)

Month	1987						1988					
	Total BSE	Total +ve	Total Pf	Slide positive rate			Total BSE	Total +ve	Total Pf	Slide positive rate		
				Infant	Child	Adult				Infant	Child	Adult
Jan	150	6	3	—	2.44	4.76	224	50	47	—	24.53	22.16
Feb	129	1	1	—	—	1.09	153	33	15	—	19.05	23.36
Mar	200	2	—	—	4.84	—	183	45	12	14.28	23.07	26.13
Apr	356	12	7	11.11	—	4.17	257	109	35	—	51.72	38.79
May	342	66	11	—	16.48	20.65	946	320	58	9.09	41.06	31.40
Jun	349	123	—	27.27	33.93	36.28	400	96	14	—	30.25	21.66
Jul	577	155	6	33.33	21.96	28.89	366	121	35	100.00	41.24	29.85
Aug	650	230	47	—	33.33	36.31	540	185	119	33.33	37.50	33.25
Sep	538	264	72	20.00	52.53	48.00	479	201	159	—	49.48	40.37
Oct	1001	473	247	14.28	49.23	47.06	255	110	104	—	46.94	42.44
Nov	616	255	215	—	52.38	38.36	104	53	47	—	43.75	54.17
Dec	400	121	110	50.00	31.64	29.65	134	62	51	—	46.94	46.99
Total	5308	1708	719	13.33	32.15	32.57	4041	1385	696	8.89	38.95	32.93

Gametocyte rate : Infant = 10.67%; Child = 20.63%; Adult = 34.31%.
Pop - 6,000; SPR - 32.18; ABER - 88.00; API -285; Pf% - 42.10.

Gametocyte rate : Infant = 8.89%; Child = 25.62%; Adult = 25.32%.
SPR - 17.22; ABER - 67.35; API - 231.00; Pf% - 50.25.

tember and then declined to low levels. In August, *P. falciparum* starts increasing, peaking in October–November and declines with the onset of winter. Although transmission in the two groups of villages was high it was difficult to say that rice fields were responsible for maintaining high levels of transmission in the study areas.

Our studies have shown that irrigation systems connected with rice fields produce *A. culicifacies* for the first 6—8 weeks of rainy season. Subsequently, although rice fields do not support *A. culicifacies,* the vector mosquito breeds continuously in stagnant water. Besides, *A. fluviatilis* breeds throughout the period of rice cultivation. The two vectors together contribute significantly to the already high vector population of the area thus enhancing transmission.

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Ultrastructural Comparison of Erythrocytic Stages of Experimentally Selected Drug Resistant Strains of Rodent Malaria Parasite *P. berghei* with its Susceptible Strain

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Studies on ultrastructure of drug-resistant strains of *Plasmodium berghei* have been mostly restricted to the experimentally selected chloroquine and pyrimethamine resistant strains. Study of the morphology of mefloquine and quinine resistant strain of *P. berghei* has been carried out to demonstrate some differences between normal and drug-resistant strains. The main differences are concerned with a complex physiological process i.e., food ingestion and digestion. E.M. studies reveal that in the sensitive strain of *P. berghei* the trophozoites possess numerous rod-shaped malarial pigment particles which lie within a vesicle. In the trophozoites of resistant parasites the malarial pigment particles are rarely visible. Trophozoites of the sensitive strain have only one large food vacuole while the trophozoite of the resistant parasite contains 2-3 smaller vacuoles.

INTRODUCTION

Several excellent reviews of the progress made in ultrastructural studies of erythrocytic stages of malarial parasites have been published by Garnham (1967), Rudzinska and Vickerman (1968), Rudzinska (1969) and Aikawa (1971).

Most ultrastructural studies on the malaria parasite have been limited to the sensitive strain. Only a few studies had been carried out on the ultrastructural changes in the morphology of the malarial parasite-induced by drug resistance. Peters *et al.* (1965) demonstrated that marked deficiency of haemozoin granules associated with presence of multiple food vacuoles was the hallmark of chloroquine resistant strain. Several other authors have also reported their views on the relationship between the presence of pigment granules and multiple food vacuoles and drug resistance.

The present paper describes the ultrastructural studies on erythrocytic stages of susceptible as well as drug-resistant strains of *P. berghei.*

MATERIAL AND METHODS

Swiss mice were inoculated with $2x10^7$ parasitised RBC i/p with sensitive and resistant strains of *P. berghei* respectively. Blood was drawn in syringe by cardiac puncture at 20-30%

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parasitaemia. The blood was allowed to clot and fixed (Hoyer *et al.*, 1979). The samples were post-fixed according to the method of Caulfield (1957). The samples were processed for electron microscopy (Mollenhauer, 1964). The sections were cut and stained (Watson, 1958; Reynolds, 1962) and grids were examined under electron microscope (Phillips 410 LS).

The following strains were used in this study:

(1) Sensitive strain of *P. berghei*
(Curative dose)
Mefloquine 4 mg/kg x 4 days
Quinine 300 mg/kg x 4 days

(2) Mefloquine resistant *P. berghei*
(Resistant to 256 mg/kg Mefloquine x 4 days)

(3) Quinine resistant *P. berghei*
(Resistant to 300 mg/kg
Quinine x 4 days; Kazim *et al.*, 1986).

RESULTS

Ultrastructure of sensitive strain of *P. berghei*

(1) *Trophozoite*: The uninucleate trophozoite is oval to round and is surrounded by a single unit membrane (Figs. 1, 2).

The mitochondria are oval or elongated with indistinguishable cristae. Ribosomes are diffusely distributed in the cytoplasm of the parasite and polyribosomes are also observed occasionally. Endoplasmic reticulum is present but is scanty and mostly of the rough type. The food vacuoles, formed by the process of phagocytosis, contain red cell cytoplasm and are usually surrounded by a unit membrane. As the digestion takes place, clusters of amorphous pigment granules appear within the cytoplasm of the parasite. The malarial pigment particles are electron dense and have crystalloid appearance. The Golgi apparatus is conspicuous and composed of small vesicles on both sides of the lamella. A number of trophozoites show proliferation of multilamellate whorls of membrane which vary from tight coils to loosely organised fascia.

(2) *Schizont*: As the trophozoite grows, its nucleus divides and small protruberances are formed on the parasite's surface. At the same time while early nuclear division takes place in the preschizont, an extra layer of merozoite membrane is laid down in the apical region of the budding merozoites. Subsequent changes in these two membranes are thought to give rise to the apical rings and conoid of the fully formed merozoite. This process was followed by the formation of the specialized organelles of the merozoite, the paired organelles (Fig. 3). At the same time pigment was observed to concentrate at one area in the developing schizont to form the residual body.

A mature schizont contains several merozoites with a residual body lying in between the merozoites (Fig. 4).

(3) *Merozoite*: The merozoite is a pear shaped structure. It is bounded by a pellicular complex. The pellicle is composed of outer and inner membranes. At the anterior end of the merozoite the inner membrane is slightly thick. The merozoite possesses a nucleus and various cytoplasmic organelles. Nucleus is large usually located at the posterior part of the merozoite and is surrounded by a double nuclear membrane. A distinct nucleolus is not seen in the nucleus of the erythrocytic merozoite. The apical end of the merozoite is truncated and two electron dense rhoptries and several micronemes are present in this region (Fig. 4).

Ultrastructure of resistant parasites

(1) *Mefloquine resistant strain*

Trophozoite: The uninucleate trophozoite is oval to round and is surrounded by a unit membrane. The nucleus is generally homogenous and ap-

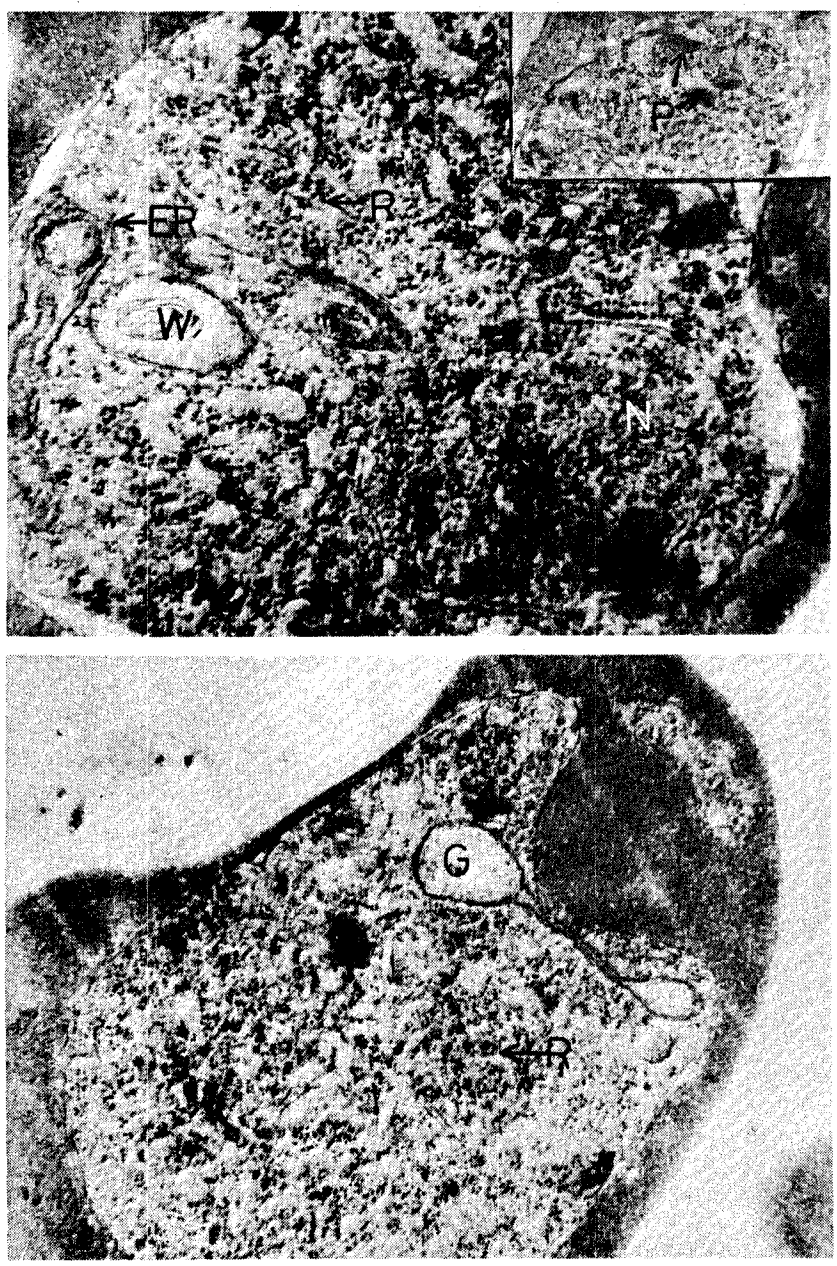
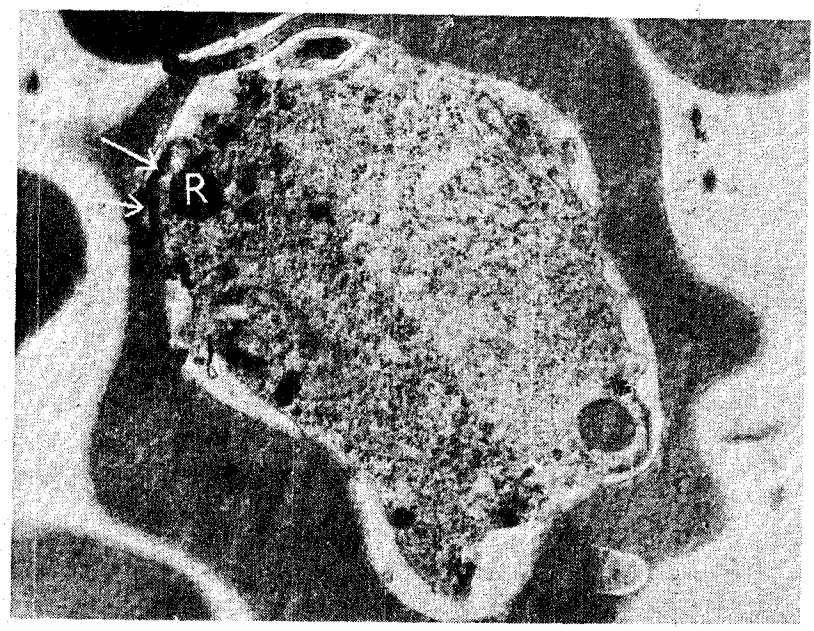


Fig. 1: Trophozoite of sensitive strain of *P. berghei* x 46,500.
N—Nucleus; W—Whorl membraned organelle; ER—Endoplasmic reticulum; R—Ribosomes.
Inset Fig. 1. Part of the trophozoite showing pigments (P) x 30,000.

Fig. 2: Trophozoite of sensitive strain of *P. berghei* x 6,200.
G—Golgi apparatus; R—Ribosomes.



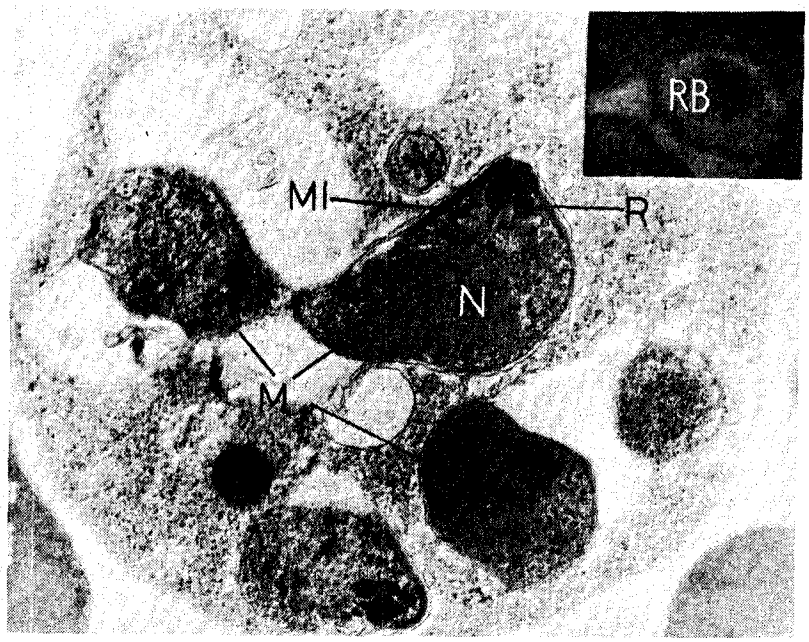


Fig. 3: Preschizont stage of *P. berghei* x 33,800.
R—Precursors of rhoptries.
Thickening of the inner membrane to form the apical region of merozoite (Arrow).

Fig.4: Schizont of sensitive strain of *P. berghei* x 33,800.
M—Merozoites; N—Nucleus; R—Rhoptries; MI—Micronemes.
Inset Fig. 4. Part of the schizont showing residual body (RB) x 40,000.

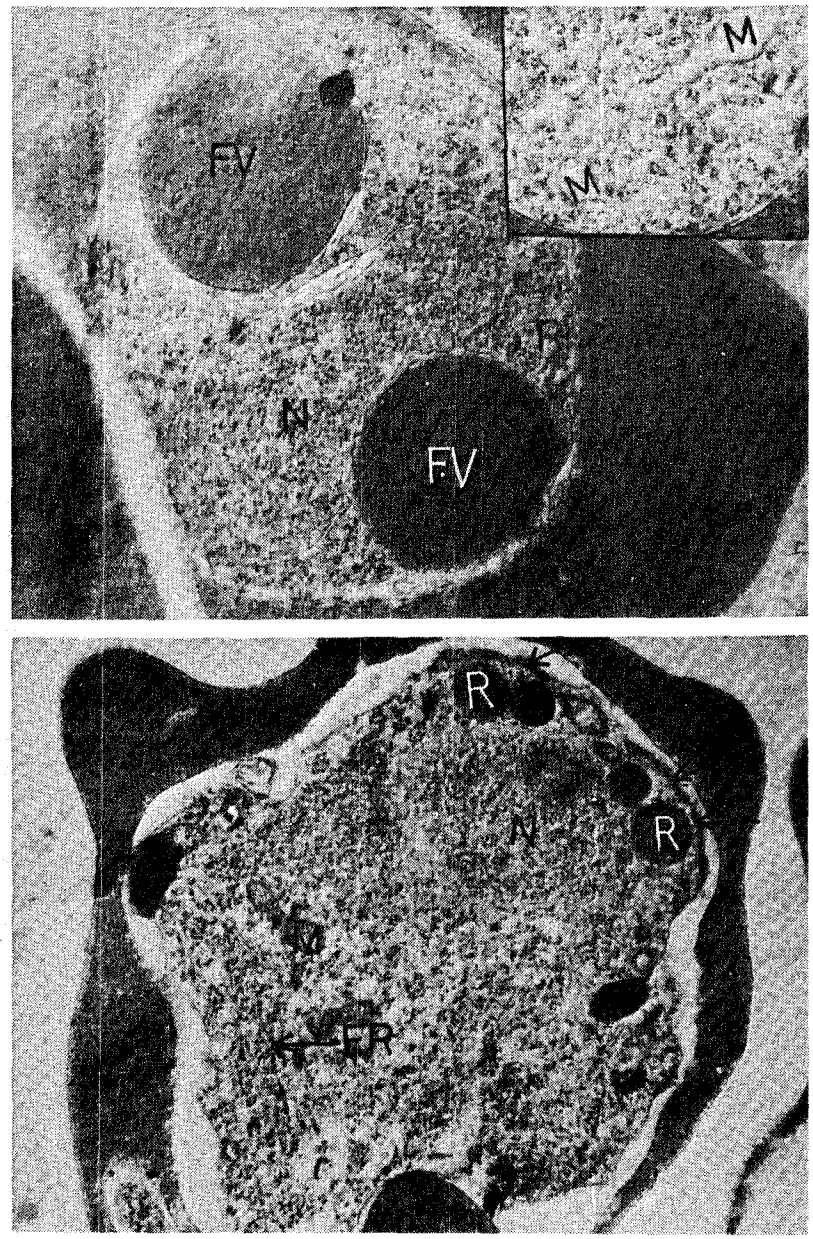
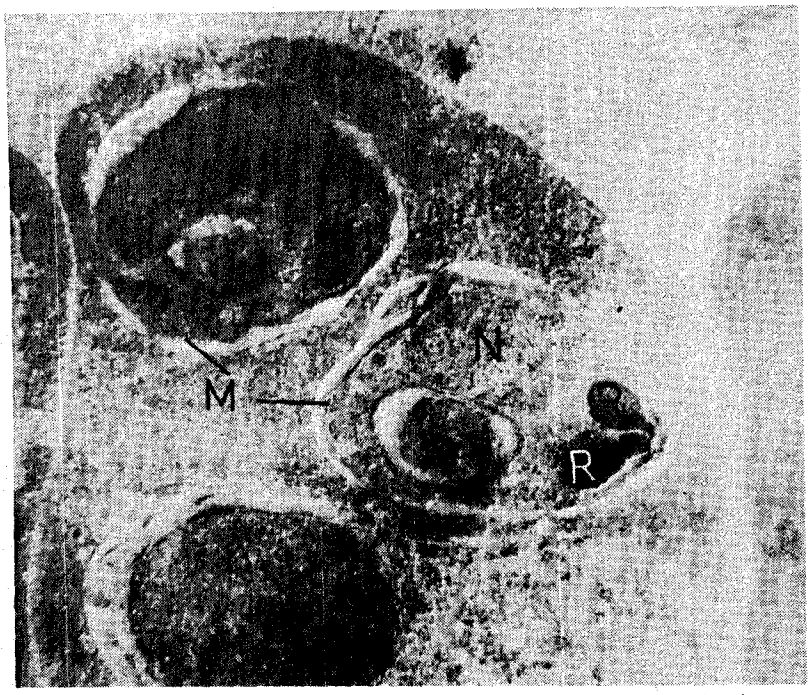


Fig. 5: Trophozoite of mefloquine resistant strain of *P. berghei* x 42,000.
FV—Food vacuole; N—Nucleus; R—Ribosomes.
Inset Fig. 5. Part of the cytoplasm of trophozoite showing mitochondrion (M) x 42,000.

Fig. 6: Preschizont of mefloquine resistant strain of *P. berghei* x 33,800.
N—Nucleus; R—Precursors of rhoptries; ER—Endoplasmic reticulum;
M—Mitochondria.
Showing thickening of apical region of merozoite.



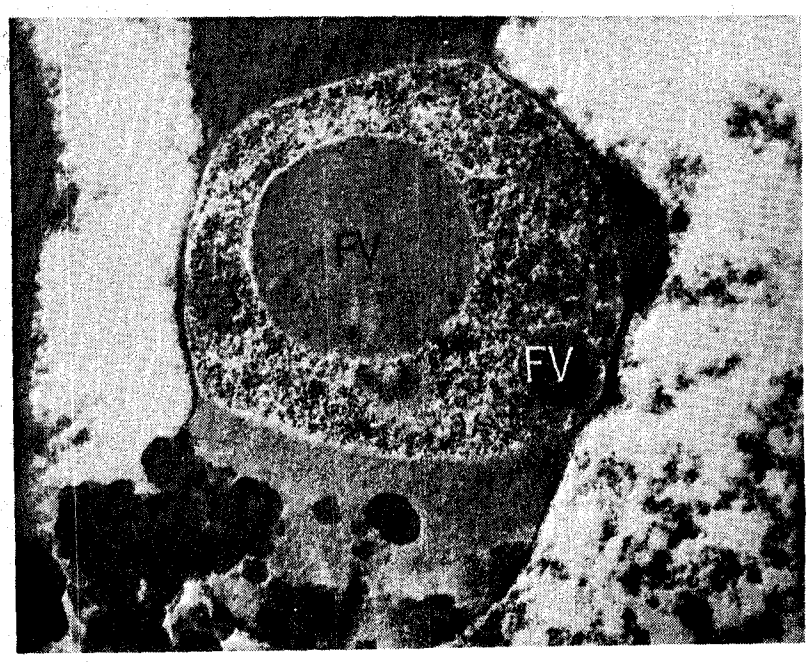


Fig. 7: Schizont of mefloquine resistant strain of *P. berghei* x 33,800. M—Merozoites; N—Nucleus; R—Rhoptries.

Fig. 8: Trophozoite of quinine resistant strain of *P. berghei* x 25,350. FV—Food vacuole.

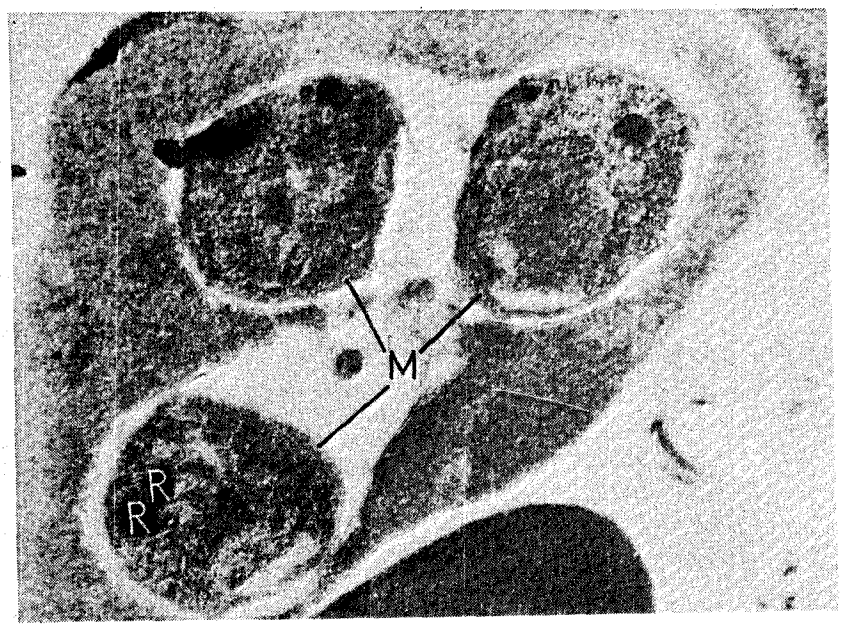


Fig. 9: Schizont of quinine resistant *P. berghei* x 31,500.
M—Merozoite; R—Rhoptries.

pears to be largely made up of euchromatin. The nucleus is surrounded by a double membrane and no distinct nucleolus is present (Fig. 5).

The mitochondria are oval or elongated and devoid of prominent cristae. A number of trophozoites possess multilamellate whorls of membranes. Ribosomes are diffusely distributed in the cytoplasm of the parasite and sometimes polyribosomes are also present.

In the trophozoite of a resistant parasite, there is a marked deficiency of haemozoin granules associated with the presence of multiple food vacuoles. The food vacuole contains red cell cytoplasm and is surrounded by a single unit membrane. The food vacuoles are formed by the process of phagocytosis. In some trophozoites, malarial pigment granules are present but they are reduced in size. The Golgi apparatus is not seen in the trophozoites of resistant parasite (Fig. 5).

Schizont: With the subsequent growth of the trophozoite the nucleus divides and budding of the cytoplasm occurs. The first sign of schizogony, is the appearance of the intranuclear mitotic spindle beneath the nuclear envelope.

Within the dividing schizont small discs of discontinuous double membrane develop beneath the plasmalemma. Beneath these discs lies a single membrane bound vesicle with an electron dense matrix. This is the first stage in the development of rhoptry-microneme complex of merozoite (Fig. 6).

A mature schizont consists of a number of merozoites surrounded by a unit membrane. In the schizont of the resistant parasite residual body is not apparent (Fig. 7).

Merozoite: The merozoite of the resistant parasite resembles that of normal parasite. It is a pear-shaped structure surrounded by a single unit membrane. The inner membrane of the merozoite is slightly thick at the anterior end of the merozoite. The merozoite possesses a single large nucleus and various cytoplasmic organelles. Nucleus is located at the posterior part of

the merozoite and is surrounded by a double nuclear membrane. Nucleolus is not apparent. The apical end of the merozoite is a truncated cone shaped structure. Two electron dense elongated structures, the rhoptries are present. In some merozoites, micronemes are also seen which are also a part of the apical complex. Cytostome is not apparent.

Mitochondria are not visible in the cytoplasm. Ribosomes are abundant and a scanty endoplasmic reticulum is seen. Golgi apparatus and spherical body is not seen (Fig. 7).

(2) *Quinine resistant P. berghei*

The most remarkable feature of the quinine resistant *P. berghei* is the presence of multiple food vacuoles with loss of malarial pigment granules as in mefloquine resistant *P. berghei* (Fig. 8).

The preschizont stage in quinine resistant *P. berghei* was also found and resembles that of mefloquine resistant parasite. The schizont contains several merozoites with prominent apical complex (Fig. 9).

DISCUSSION

The present study demonstrates some differences between normal and drug-resistant strains. The main differences are concerned with a complex physiological process i.e., food ingestion and digestion. EM studies reveal that in the sensitive strain the trophozoites possess numerous malarial pigments. The malarial pigment particles are rod-shaped structures and lie within a vesicle. In the trophozoites of resistant parasites the malarial pigment particles are absent. In rare cases haemozoin granules are present in the cytoplasm of the trophozoite but they are coarse and smaller in size as compared to that in sensitive strain.

The second difference is with regard to the number of food vacuoles present in individual trophozoites. The trophozoite of the sensitive strain has only one large food vacuole but that of the resistant parasite contains 2-3 vacuoles which are smaller in size. The schizont of the sensitive strain of *P. berghei* has a residual body having several pigment granules while the schizont of resistant strain has no residual body.

The above observation is in agreement with the earlier observations of Peters (1970). During the course of his studies with chloroquine resistant strain of *P. berghei*, Peters (1965) observed a number of morphological differences between intraerythrocytic stages of the normal and resistant strains. Very few typical schizonts were visible during the first two weeks but as the infection progressed after this time many appeared in the peripheral circulation and these were remarkable for a complete absence of the usual clumped pigment in the residual bodies. Rabinovitch (1965) observed similar changes in chloroquine resistant and in cycloguanil resistant strains of *P. berghei*. Under phase contrast, living parasites of the two strains were examined and in normal strain the grains of haemozoin showed up as clearly defined refractile bodies in a single food vacuole. In chloroquine resistant parasite, no refractile bodies were seen and multiple food vacuoles were present in many trophozoites.

Electron microscope studies confirmed these observations and the marked deficiency of haemozoin granules associated with the presence of multiple food vacuoles was shown to be the hallmark of a chloroquine resistant strain (Peters *et al.*, 1965). Peters has attempted to correlate the parasite ultrastructure with the age of the host cell since it is well known that less pigment is formed even by a normally sensitive *P. berghei* trophozoite when it grows in a polychromatic red cell. Although Howells *et al.* (1968) have shown clearly that there is a tendency for smaller haemozoin particles to be formed even in normal strains of parasite that develop within immature red cells, the reduction on the whole is less marked than observed in the chloroquine resistant strain while normal strain

does not form the multiple food vacuoles that are so typical of the resistant parasites.

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SHORT NOTES

Indian Journal of Malariology
Vol. 26, December 1989, pp. 211-214.

Control of Mosquito Breeding in Wells by the Application of Expanded Polystyrene (EPS) Beads

S.N. TIWARI[1] and P.K. TYAGI[1]

Wells are an important site of mosquito breeding in both rural and urban areas. In most areas a large number of wells are unused or abandoned while some are used for irrigation or used occasionally and only small numbers are used regularly, mostly for drawing water for daily needs. It has been observed that mosquito breeding is more frequently encountered in unused/abandoned wells than in wells which are frequently used. Although chemical and biological control methods have been suggested to control mosquito breeding in wells, the application of expanded polystyrene (EPS) beads is a promising and practical method particularly for unused wells (Chandrahas and Sharma, 1987). Results of a one year study on the control of mosquito breeding in wells of Shahjahanpur and Allahabad district villages are reported in this paper.

The experimental wells were located in an area under the bioenvironmental control of malaria project in Dadrol block (Shahjahanpur district) and Shankargarh block (Allahabad district). The target area comprised wells in 26 experimental and 5 control villages in Dadrol block and 10 experimental and 3 control villages in Shankargarh block. All wells of the study area of Dadrol and Shankargarh block were surveyed in the month of March 1987 and 1988 respectively, and divided into three groups i.e., "used", "occasionally used" and "unused". Out of unused wells in the experimental areas 15 wells were positive (9 for culicines, 2 for anophelines and 4 for mixed breeding) in Dadrol block and 33 wells were positive (5 for culicines, 22 for anophelines and 6 for mixed breeding) in Shankargarh block. All these wells were treated with expanded polystyrene (EPS) beads @ 85 gm/ 1000 cm^2. Wells in Dadrol block were treated with EPS in April 1987. In Shankargarh block 7 wells were treated in April 1988; 12 in June 1988 and 14 in October 1988. Three unused wells in Dadrol and 5 in Shankargarh were kept as controls.

Pre- and post-treatment density of immatures was measured by taking dips using a galvanized bucket (5 lit. capacity). A total of 5 dips were taken from each well. Breeding density was calculated by taking an average of 5 dips per well. The results in the table are based on the average number of I-III instars, IV instars and pupae per well. Even if one larva or pupa was found, the well was considered positive. The anophelines and culicines were categorised as I-III instar, IV instar and pupae. Post-treatment observations were carried out at weekly intervals for 8

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months in Dadrol and for 12 months in Shankargarh block. For brevity, data is presented for the first three weeks followed by average number of immatures per dip at monthly intervals.

Table 1 gives the results of mosquito breeding surveys in wells of Dadrol block. Following the application of EPS beads there was drastic decline in wells positive for mosquito immatures in successive weeks. Out of 15 wells, 8 wells became negative within one week of sealing with beads. Of the remaining seven wells, three became negative in two weeks, two within three weeks and two in the fourth week. During the 8 month observation period all wells remained negative for mosquito breeding, whereas wells held as control were found positive throughout the period of observation.

Table 2 gives the results of wells in Shankargarh block. Larval density was gradually reduced and reached zero in the first week in 24 wells. The remaining wells became free of mosquito immatures in the second (4 wells), third (2 wells) and fourth (3 wells) weeks respectively. All 33 wells remained negative for mosquito immatures for

Table 1. Impact of EPS beads on mosquito breeding in wells of Dadrol PHC, Shahjahanpur district (U.P.)

	Immature density/Dip					
	Experimental wells-15			Control wells-3		
	I-III	IV	Pupae	I-III	IV	Pupae
Day 0	213.78 (9.6-1161.2)	13.8 (0.2-81.4)	3.3 (0-28.6)	91.6 (12-243)	3.3 (0-9)	0
Week-I	14.5 (0-119.8)	0.8 (0-6)	0	12.3 (4-20)	0	0
Week-II	0.5 (0-5.2)	0	0	18 (2-30)	1 (0-3)	0
Week-III	0.1 (0-1.4)	0	0	18 (7-40)	0.3 (0-1)	1.3 (0-4)
Month-1	0	0	0	12.7 (10-17)	3 (1-6)	1.7 (0-4)
Month-2	0	0	0	10 (7-13)	2.3 (0-5)	0
Month-4	0	0	0	13.3 (5-28)	1.3 (0-4)	2 (0-4)
Month-6	0	0	0	62 (2-121)	6.3 (0-15)	2.3 (0-6)
Month-8	0	0	0	122.3 (8-258)	5 (0-8)	5.6 (0-11)

Figures in parentheses indicate the range of immature density in wells.

Table 2. Impact of EPS beads on mosquito breeding in wells of Shankargarh block, Allahabad district (U.P.)

	Immature density					
	Experimental wells-33			Control wells-5		
	I-III	IV	Pupae	I-III	IV	Pupae
Day 0	136.06 (2-3426)	16.74 (0-341)	1.2 (0-12)	20.0 (13-52)	1.6 (0-7)	0
Week-I	6.89 (0-153)	0.52 (0-9)	0	67.6 (7-277)	13.0 (0-62)	8.6 (0-43)
Week-II	0.56 (0-6)	0.09 (0-2)	0	50.2 (8-210)	4.4 (0-20)	7.6 (0-38)
Week-III	0.15 (0-3)	0	0	13.4 (0-50)	4.8 (0-24)	11.4 (0-54)
Month-1	0	0	0	11.0 (0-28)	2.4 (0-12)	0
Month-2	0.45 (0-15)	0	0	5.8 (2-16)	1.4 (0-7)	0.4 (0-2)
Month-4	0	0	0	4.8 (4-10)	2.0 (0-8)	0.6 (0-3)
Month-6	0	0	0	168.8 (7-825)	3.4 (0-10)	0.6 (0-3)
Month-9	0*	0*	0*	124.0 (4-610)	1.8 (0-7)	2.6 (0-13)
Month-12	0**	0**	0**	37.0 (17-141)	4.4 (0-19)	0.8 (0-2)

*Average of 19 wells; **Average of 7 wells; Figures in parentheses indicate the range of immatures in dips.

12 months observations. However, in one well breeding reappeared 2 months after the introduction of beads because of disturbance in the layer. Some more beads were applied in this well which again became negative. Density of immatures in control wells fluctuated considerably but none of the control wells became negative during the observation period.

The idea of sealing the water surface with EPS beads to control *Culex quinquefasciatus* was first suggested by Reiter in 1978. Laboratory trials by Sharma (1984) showed that application of EPS beads @ 1 gm per 500 ml beaker produced high larval and pupal mortality in *Culex quinquefasciatus, Anopheles culicifacies, A. stephensi* and *Aedes aegypti* and prevented mosquitoes from laying eggs on treated surface. A field study in Kheda district (Gujarat), demonstrated that application of EPS beads is an effective method for the control of mosquito breeding in wells and biogas plants (Sharma *et al.*, 1985). At other places EPS beads have been used to control mosquito breeding in soakage pits, pit latrines and sluice valve chambers (Curtis and Minjas, 1985; Dua *et al.*, 1989). A small-scale field trial in and around Delhi showed that EPS beads were successful on a long-term basis for the control of

mosquito breeding in overhead tanks and disused wells (Chandrahas and Sharma, 1987). Our experiments in the control of mosquito breeding in disused wells in rural areas of Shahjahanpur and Allahabad villages clearly confirmed the usefulness of EPS beads in the elimination of mosquito breeding in wells. The technique is now being applied to the entire districts of Shahjahanpur and Allahabad besides other field stations in experimental areas for the control of mosquito breeding in wells and similar other habitats.

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Indian Journal of Malariology
Vol. 26, December 1989, pp. 215-218.

Laboratory Diagnosis of Malaria Infection and its Natural History in an Urban Pocket of Hyderabad City

M.M.A. KHAN[1], M.A. KAREEM[1] and G.K. RAO[1]

A study was conducted in an urban pocket of Hyderabad City on the prevalence of malaria, breeding of the vector *A. stephensi* and to cross check the methodology of screening fever cases for detection of malaria parasites. Study revealed the need to emphasize laboratory diagnosis of malaria even in the absence of history of pyrexia and to carry out radical treatment in case of *Plasmodium vivax* infection. There are indications that malaria morbidity was high in the area and required effective implementation of anti-malaria operations in the city.

A malaria survey was undertaken in the Family Health Care Programme under the Department of Social and Preventive Medicine of Deccan College of Medical Sciences, Hyderabad. The Ward 18 of Municipal Corporation of Hyderabad situated on the south of Moosi river in Hyderabad City was taken up for the study, which revealed the presence of malaria infection in August 1987. Thirty (30) blood smears were then collected out of which one was positive for *P. vivax* in a girl of 4 years age. This prompted the present study which attempts to correlate epidemiological and environmental factors in the area and its neighbourhood, while assessing the malaria morbidity.

The morbidity pattern in the population in relation to malaria and its symptomatology, mainly history of pyrexia, was elicited by a team consisting of a male and a female doctor of the Department of Social and Preventive Medicine of Deccan College of Medical Sciences, Hyderabad, with the help of paramedical personnel. Simultaneously, blood smears were collected by finger prick method and thick and thin smears were made for detection of malarial parasite after staining with JSB. From the study population, consisting of 146 individuals in 24 families, in a compact area of ward 18 (G.M. Nagar), 112 blood smears were collected as part of mass blood survey during a three month period ending August 1989. From the remanent group of individuals (23.3%) of the total population under study, blood smears could not be collected because of a variety of reasons (mainly on account of change of their residence) Passive case detection (PCD) was also carried out in the Out-Patient Department (OPD) of the Medical College, for which a field worker was deputed by the malaria department of the Directorate of Medical and Health Services of Govt. of Andhra Pradesh (for the collection of blood smears from fever cases). All malaria positive cases were administered the prescribed radical treatment.

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Data pertaining to cases during March to August 1989 were also collected from 15 private medical practitioners' clinics and 2 nursing homes and a laboratory diagnostic centre in the area and its neighbourhood by a medico-social worker of the Department. This data was obtained as per a questionnaire. An entomological survey, limited in scope, was also taken up to determine the presence of malaria vectors in the area.

Results of blood smears collected from 112 individuals of 24 families in the study area, revealed 21 (among them an infant) positive cases, all *Plasmodium vivax.* Results of PCD from the OPD from June to August 1989 showed that out of 307 blood smears collected 160 were positive; 159 for *P. vivax* and 1 for *P. falciparum* (gametocytes). The results are depicted in Table 1 according to age groups and show a slide positivity rate of 18.8% for mass blood survey and 52.1% for PCD. Table 2 presents wardwise distribution of malaria positive cases amongst PCD. It was observed that malaria was prevalent not only in the area of study i.e., G.M. Nagar but also in the neighbouring areas of Phisalbanda, Riyasathnagar and Santoshnagar in wards 17 and 18. Since all the individuals in this study were subjected to mass blood survey, the slide positivity rate (SPR) of 18.8% equals and represents the point prevalence rate in this traditional malariometric survey (Swaroop, 1966).

Information collected by the medico-social worker of the Department revealed that of the private medical agencies covered by the survey, only 5 institutions were undertaking laboratory diagnosis for malaria. Total estimated outpatients' attendance of the 17 private medical institutions during six months was 67,680; out of which 17,083 were fever cases. In five institutions where laboratory diagnosis was undertaken, 7,617 fever cases were recorded and 1,390 were positive for malaria parasite in the laboratory diagnosis, giving a slide positivity rate of 18.3% (much less than the slide positivity rate of 52.1% obtained in the passive case detection at the outpatient department of the college). Data revealed that there was a lot of overt malaria morbidity coming to the surface. Table 3 gives relationship between the history of pyrexia and laboratory diagnosis of malaria. Its validity as a screening procedure for subjecting the individual for laboratory diagnosis is found low, as evi-

Table 1. Agewise distribution of malaria cases in the study population and medical college out-patient PCD

Age groups (yrs)	Blood smears collected (Jun-Aug 1989)			
	Mass Blood Survey	Positive/ Species	PCD	Positive/ Species
0-1	8	1/Pv	1	1/Pv
2-4	19	4/Pv	21	11/Pv
5-14	30	4/Pv	81	44/43Pv+ 1 Pf (g)
14 and above	55	12/Pv	204	104/Pv
Total	112	21/Pv	307	160 159 Pv + 1 Pf (g)

PCD—Passive Case Detection; Pv—*Plasmodium vivax*; Pf(g)—*Plasmodium falciparum* (gametocyte).
Note: Slide positivity rate (SPR) for MBS was 18.8% and for PCD 52.1%.

Table 2. Areawise distribution of malaria cases, of the medical college out-patient department

Area	Ward/block	Positives(%)	Species
Phisalbanda	18-8	37 (23.1)	Pv
G.M. Nagar*	18-14	19 (11.9)**	Pv
Riyasathnagar	17-8	36 (22.5)	Pv
Santoshnagar	17-1	20 (12.5)	Pv
Dargah Barnah Shah	17-1	11 (6.9)	Pv [10 Pv + 1 Pf (g)]
Other areas	16-24	37 (23.1)	Pv
		160 (100.0)	

*Study area is a part of G.M. Nagar.
**The cases are not from the study population.

Table 3. Validity of history of pyrexia with regard to the diagnosis of malaria in the study population

		Lab. diagnosis of malaria		Total
		+ve	−ve	
History of pyrexia	Present	12(a)	30(b)	42
	Absent	9(c)	61(d)	70
Total		21	91	112(N)

Sensitivity = a/a + c = 57.14%; False −ve rate = c/N = 8.04%.
Specificity = d/b + d = 67.03%; False +ve rate = b/N = 26.79%.

denced by its sensitivity and specificity of 57.14% and 67.03% respectively. This screening procedure would miss 42.86% of true positive cases, resulting in a false-negative rate of 8.03% which would continue to be a hidden reservoir of infection. The false-negative rate of 8.03% represents the well-known phenomenon of asymptomatic parasitaemia in *Plasmodium vivax* where relapses are common. The infection is difficult to cure due to the fact that treatment in such infections is carried out mostly by private practitioners by way of presumptive treatment and radical cure is seldom instituted. However, more studies are required on the phenomenon of asymptomatic parasitaemia in *P. vivax* cases.

Due to paucity of trained personnel, the survey was limited to collection of larvae from breeding places. With the onset of monsoons in June 1989 a large number of water collections were observed in G.M. Nagar Colony adjoining the college. Kanchanbagh area included in this study also contained many ornamental fountains and cisterns in the palace of the former Nawab of Paigah. Larvae and pupae collected from cisterns and fountains in July 1989 were reared in the laboratory. Both *A. stephensi* and *Ae. aegypti* emerged from the collections. Several studies in the past have shown that *A. stephensi* was responsible for malaria transmission in Hyderabad City (Abraham, 1932; Anon, 1957; Sitara-

man *et al.*, 1975; Rao, 1984). It is evident from the preceding data that various epidemiological factors of the disease including environmental conditions are existing in certain blocks of Municipal Wards 17 and 18, culminating in this situation. Further, the high slide positivity rate and the positivity of infant blood smear, coupled with the finding of active vector breeding in the area, are conclusive evidence of active transmission of the disease.

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Corrigendum

IJM 26(2) June 1989

Efficacy of 5 Day Radical Treatment of Primaquine in *Plasmodium vivax* Cases at the BHEL Industrial Complex, Hardwar (U.P.) by Sinha *et al.*

Page 84—Fig. 1

Legends should be read as

"X - axis Year

Y - axis Number of vivax cases."

Page 85 under Results and Discussion

The first sentence should be read as

"Blood smear examination (during September to December 1986, January to December 1987 and till March 1988) revealed 763, 569 and 13 cases of *P. vivax*."

INDIAN JOURNAL OF MALARIOLOGY

Editorial Acknowledgement

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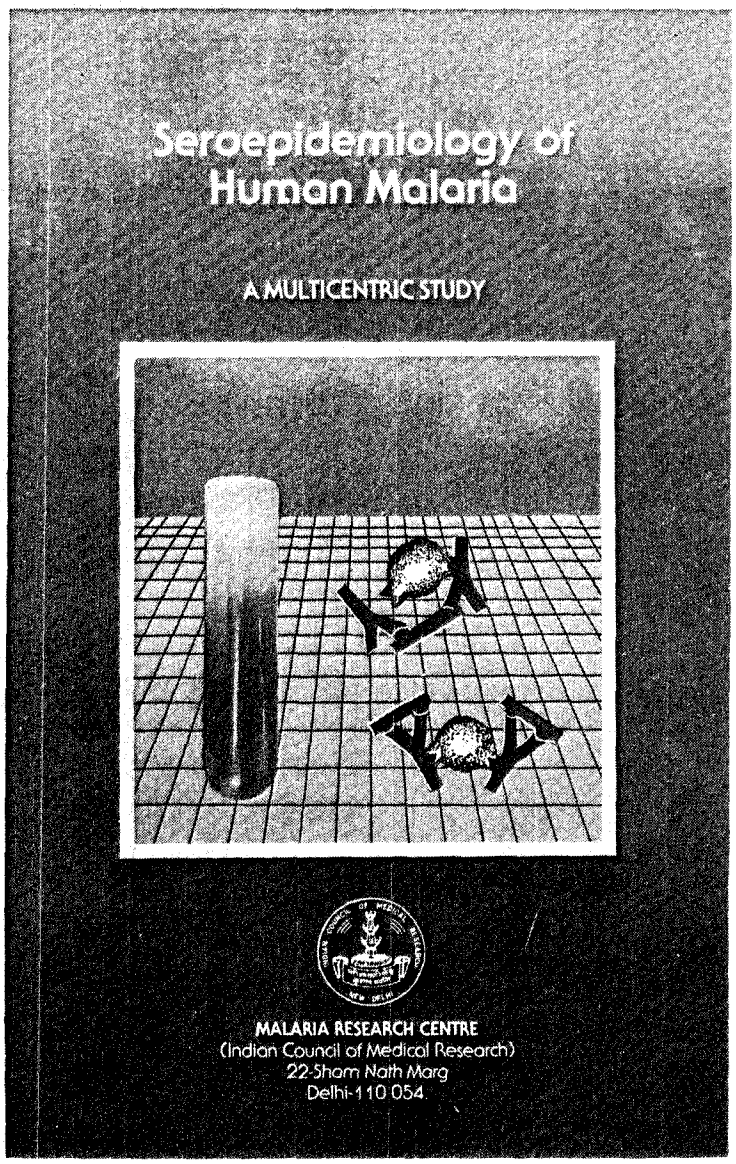

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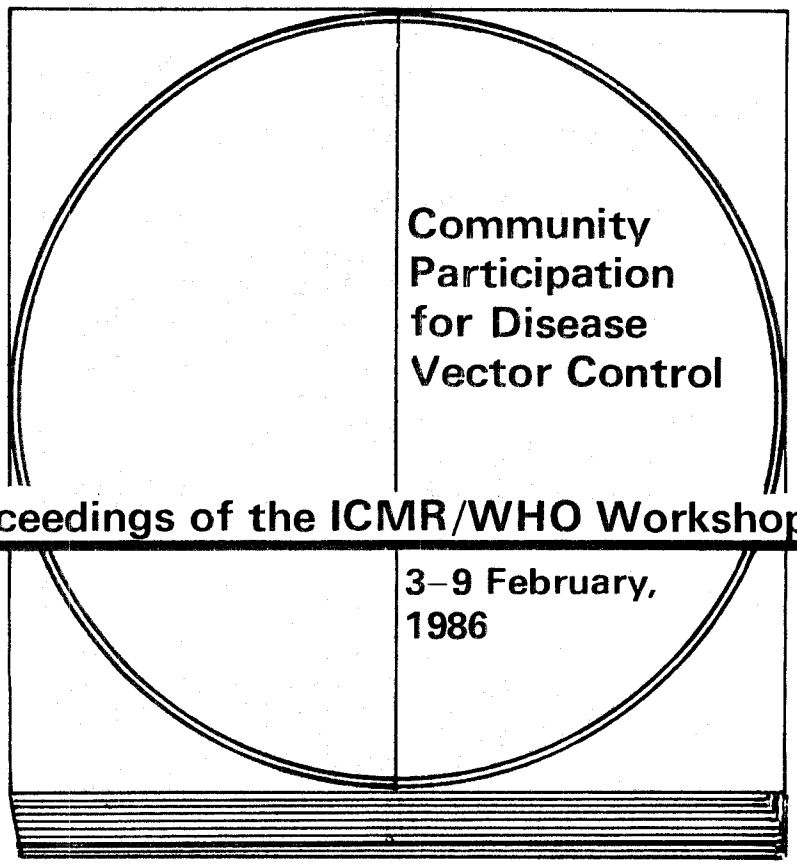

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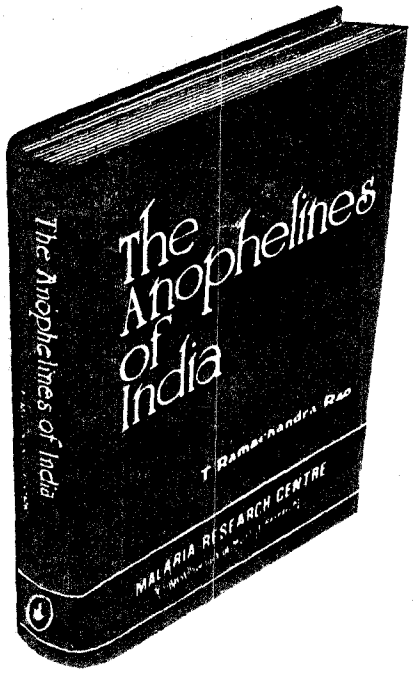

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